

# *Acinetobacter* spp. as Nosocomial Pathogens: Microbiological, Clinical, and Epidemiological Features

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## INTRODUCTION

The control of hospital-acquired infection caused by multiply resistant gram-negative bacilli has proved to be a particular problem over the last 20 years in developed countries. An increasing incidence during the 1970s of resistant members of the family *Enterobacteriaceae* involved in nosocomial infections was followed by the therapeutic introduction of newer broad-spectrum antibiotics in hospitals and a subsequent increase in the importance of strictly aerobic gram-negative bacilli, including *Pseudomonas aeruginosa*, *Stenotrophomonas (Xanthomonas) maltophilia*, and *Acinetobacter* spp.

Of these "newer" pathogens, it is now recognized that *Acinetobacter* spp. play a significant role in the colonization and infection of patients admitted to hospitals. They have been implicated in a variety of nosocomial infections, including bacteremia, urinary tract infection, and secondary meningitis, but their predominant role is as agents of nosocomial pneumonia, particularly ventilator-associated pneumonia in patients confined to hospital intensive care units (ICUs). Such infections are often extremely difficult for the clinician to treat because of the widespread resistance of these bacteria to the major groups of antibiotics. Various mechanisms of antibiotic resistance have been recognized in these bacteria, and combination therapy is usually required for effective treatment of *Acinetobacter* nosocomial infections. These therapeutic difficulties are coupled with the fact that these bacteria have a significant capacity for long-term survival in the hospital environment, with corresponding enhanced opportunities for transmission between patients, either via human reservoirs or via inanimate materials.

Despite the increasing significance and frequency of multiply resistant *Acinetobacter* infections, many clinicians still lack an appreciation of the potential importance of these organisms in hospitals, in part because of the confused taxonomic status which, until recently, was associated with these organisms. However, new molecular identification and typing methods for members of this genus have now been developed, and these should form a rational scientific foundation for proper epidemiological studies of genotypically related strains involved in outbreaks of hospital infection. Although some rare cases of community-acquired infections caused by *Acinetobacter* spp. have been reported, the primary pathogenic role of these bacteria is undoubtedly as nosocomial pathogens in hospitals. The purpose of this review is to summarize the current state of knowledge regarding *Acinetobacter* spp. in relation to this role, including their taxonomy, identification, microbiology, epidemiology, infections, and resistance to antibiotics and the potential therapeutic approaches. Interested readers are also referred to the recent thesis published by Gerner-Smidt (63).

## TAXONOMY

### Historical Features

Bacteria now classified as members of the genus *Acinetobacter* have suffered a long history of taxonomic change. The original concept of the genus *Acinetobacter* (27, 28, 151) included a heterogeneous collection of nonmotile, gram-negative, oxidase-positive, and oxidase-negative saprophytes that could be distinguished from other bacteria by their lack of pigmentation (89). Extensive nutritional studies (14) showed clearly that the oxidase-negative strains differed from the oxidase-positive strains, and in 1971, the Subcommittee on the Taxonomy of *Moraxella* and Allied Bacteria recommended that the genus *Acinetobacter* comprise only oxidase-negative strains (119). This division has been supported by the use of transfor-

mation tests (104), which have now been used for over two decades as the basis for inclusion of individual isolates within the genus.

Gram-negative, nonfermentative bacteria currently recognized as belonging to the genus *Acinetobacter* have been classified previously under at least 15 different "generic" names, the best known of which are *Bacterium anitratum* (171); *Herellea vaginicola* and *Mima polymorpha* (42); *Achromobacter*, *Alcaligenes*, *Micrococcus calcoaceticus*, and "B5W" (105); and *Moraxella glucidolytica* and *Moraxella lwoffii* (27, 151). It is only relatively recently that rational taxonomic proposals for these organisms have emerged, and delineation of species within the genus is still the subject of research (see below).

### Current Taxonomic Status

The genus *Acinetobacter* is now defined as including gram-negative (but sometimes difficult to destain) coccobacilli, with a DNA G+C content of 39 to 47 mol%, that are strictly aerobic, nonmotile, catalase positive, and oxidase negative. Good growth occurs on complex media between 20 and 30°C without growth factor requirements, while nitrates are reduced only rarely. Crucially, extracted DNA is able to transform mutant strain BD413 *trpE27* to the wild-type phenotype (104). Most *Acinetobacter* strains can grow in a simple mineral medium containing ammonium or nitrate salts and a single carbon and energy source such as acetate, lactate, or pyruvate.

*Bergey's Manual of Systematic Bacteriology* classified the genus *Acinetobacter* in the family *Neisseriaceae* (106), with one species, *Acinetobacter calcoaceticus*. This "species" has often been subdivided in the literature into two subspecies, subsp. *anitratus* (formerly *Herellea vaginicola*) and subsp. *lwoffii* (formerly *Mima polymorpha*) (81, 105), but this arrangement has never been formally approved by taxonomists. More recent taxonomic developments have resulted in the proposal that members of the genus should be classified in the new family *Moraxellaceae*, which includes *Moraxella*, *Acinetobacter*, *Psychrobacter*, and related organisms (164) and which constitutes a discrete phyletic branch in superfamily II of the *Proteobacteria* on the basis of 16S rRNA studies and rRNA-DNA hybridization assays (163, 216). Further details of the laboratory identification of members of the genus *Acinetobacter* are given in a later section.

### Delineation of Species

Traditionally, a microbial species has been considered to be a group of strains that show a high degree of similarity in terms of their phenotypic properties. However, it is now generally accepted that nucleic acid hybridization and sequencing studies provide the best available and most rational methods for designating species and determining relationships between different organisms. A formal molecular definition of a species has been proposed (224); it states that a species should include strains with approximately 70% or greater DNA-DNA relatedness and 5°C or less divergence values ( $\Delta T_m$ ). Genomic species that can be differentiated by phenotypic properties can then be given a formal species name.

Although it was known from early DNA hybridization experiments performed by a nitrocellulose filter method that the genus *Acinetobacter* was heterogeneous (92), only two species, *A. calcoaceticus* and *A. lwoffii*, were included in the *Approved Lists of Bacterial Names* (184) and only one species was described in *Bergey's Manual of Systematic Bacteriology* (106). On the basis of the DNA relatedness criteria outlined above, 19 DNA-DNA homology groups (genomic species) have now been recognized within the genus (23, 25, 138, 196), although

TABLE 1. Delineation of *Acinetobacter* genomic species

Species name <sup>a</sup>	Genomic species number according to <sup>b</sup> :			Type strain
	Bouvet et al. (23, 25)	Tjernberg and Ursing (196)	Nishimura et al. (137, 138)	
<i>A. calcoaceticus</i>	1	1	1N	ATCC 23055
<i>A. baumannii</i>	2	2	1N	CIP 70.34
UN	3	3	NT	ATCC 19004
UN	UG	13TU	NT	ATCC 17903
<i>A. haemolyticus</i>	4	4	4N	ATCC 17906
<i>A. junii</i>	5	5	NT	ATCC 17908
UN	6	6	4N	ATCC 17979
<i>A. johnsonii</i>	7	7	3N	ATCC 17909
<i>A. lwoffii</i>	8	8TU	2N	ATCC 15309
UN	9	8TU	NT	ATCC 9957
UN	10	10	UG	ATCC 17924
UN	11	11	UG	ATCC 11171
<i>A. radioresistens</i>	(12) <sup>c</sup>	12	5N	IAM 13186
UN	13	14TU	NT	ATCC 17905
UN	14	NT	NT	Bouvet 382
UN	15	NT	NT	Bouvet 240
UN	16	UG	NT	ATCC 17988
UN	17	NT	NT	Bouvet 942
UN	NT	15TU	NT	Tjernberg 151a

<sup>a</sup> UN, unnamed genomic species.

<sup>b</sup> NT, not tested; UG, ungrouped.

<sup>c</sup> Unpublished result.

there are some minor discrepancies in the numbering schemes proposed for genomic species by different laboratories (Table 1) and a definitive numbering scheme has yet to be finally agreed. Seven of the genomic species have been given formal species names, with the species *A. radioresistens* (137) being shown to be equivalent to genomic species 12 (196). Groups 1, 2, 3, and 13TU (196) have been shown to have an extremely close relationship and are referred to by some research groups as the *A. calcoaceticus*-*A. baumannii* complex (66). There is still a need for a rapid and reliable method of assigning new isolates to individual genomic species.

### Species of Clinical Importance

Numerous studies have now supported the original observation (24) that *A. baumannii* is the main genomic species associated with outbreaks of nosocomial infection. In a typical study of 584 *Acinetobacter* strains isolated from 420 patients at 12 different hospitals over a 12-month period (172), 426 (72.9%) strains were identified as *A. baumannii*, with 208 *A. baumannii* isolates being recovered from respiratory tract specimens, 113 being recovered from blood cultures and central venous lines, 70 being recovered from wound swabs, and 35 being recovered from other miscellaneous specimens (Table 2). This large study also identified 158 isolates that belonged to species other than *A. baumannii*, of which the most common were *Acinetobacter* genomic species 3 (55 isolates), *A. johnsonii* (29 isolates), and *A. lwoffii* (21 isolates).

Further investigations are required to define the clinical significance of *Acinetobacter* spp. other than *A. baumannii*. Such isolates in clinical specimens are often considered to be contaminants derived from the environment. Diagnosis of infection with "unusual" *Acinetobacter* genospecies therefore often depends on clinical indications and repeated isolation of the same strain from a single patient. *Acinetobacter* genomic species 3 and 13TU (196) have been implicated in nosocomial

outbreaks of infection (47), while *A. johnsonii* has been associated with catheter-related bacteremia (177). A study in Sweden found that *Acinetobacter* genomic species 3 was predominant among clinical isolates (196).

It is worth re-emphasizing the close relationship between genomic species 1 (*A. calcoaceticus*), 2 (*A. baumannii*), 3, and 13TU. This *A. calcoaceticus*-*A. baumannii* complex (66, 108) contains isolates that are mostly glucose acidifying, and the complex therefore corresponds quite well to the *A. calcoaceticus* subsp. *anitratu*s designation that is, regrettably, still used in some new reports. Early studies demonstrated that these organisms, identified originally as *Mima* and *Herellea vaginicola*, are members of the human skin flora (192). The majority of glucose-negative, nonhemolytic strains found in clinical specimens seem to be identified mainly as *A. lwoffii*, *A. johnsonii*, or *Acinetobacter* genomic species 12, and it seems that these species are also natural inhabitants of human skin. Most hemolytic isolates are identified as *A. haemolyticus* or *Acinetobacter* genomic species 6. Other groups seem to be implicated only occasionally in human infections.

In conclusion, although *A. baumannii* appears to be the *Acinetobacter* genomic species of greatest clinical importance, repeated isolation of another genomic species (particularly one belonging to the *A. calcoaceticus*-*A. baumannii* complex) from a patient should be a cause for suspicion of infection, especially if clinical symptoms are also present.

## LABORATORY IDENTIFICATION

### Isolation from Clinical Specimens

*Acinetobacter*s can be grown readily on common laboratory media such as nutrient agar and tryptic soy agar, although defined media consisting of a mineral base containing ammonium or nitrate salts and one or more carbon sources have been used for specific purposes (14, 23, 24, 66, 105, 108). However, for direct isolation from clinical specimens, it is more useful to use a selective medium that suppresses the growth of other microorganisms. A selective and differential medium containing bile salts, sugars, and bromocresol purple (122) is available commercially as *Herellea* agar (Difco). This has been modified by the addition of various antibiotics (85), and the addition of antibiotics to more common media has also been used in the investigation of several outbreaks of *Acineto*

TABLE 2. Distribution of *Acinetobacter* spp. isolated from clinical sources<sup>a</sup>

Genomic species	No. of isolates from:				Total no.
	Tracheal aspirate	Blood culture and central venous line	Wound swab	Other sites	
<i>A. baumannii</i>	208	113	70	35	426
Sp. 3	6	24	10	15	55
<i>A. johnsonii</i>	0	19	2	8	29
<i>A. lwoffii</i>	2	12	2	5	21
<i>A. junii</i>	0	7	0	4	11
<i>A. haemolyticus</i>	0	1	2	6	9
Sp. 10	0	6	1	2	9
Sp. 11	1	0	1	2	4
Sp. 12	0	3	0	0	3
Sp. 6	0	1	0	0	1
Unidentified	1	5	2	8	16
Total	218	191	90	85	584

<sup>a</sup> Data from reference 172.

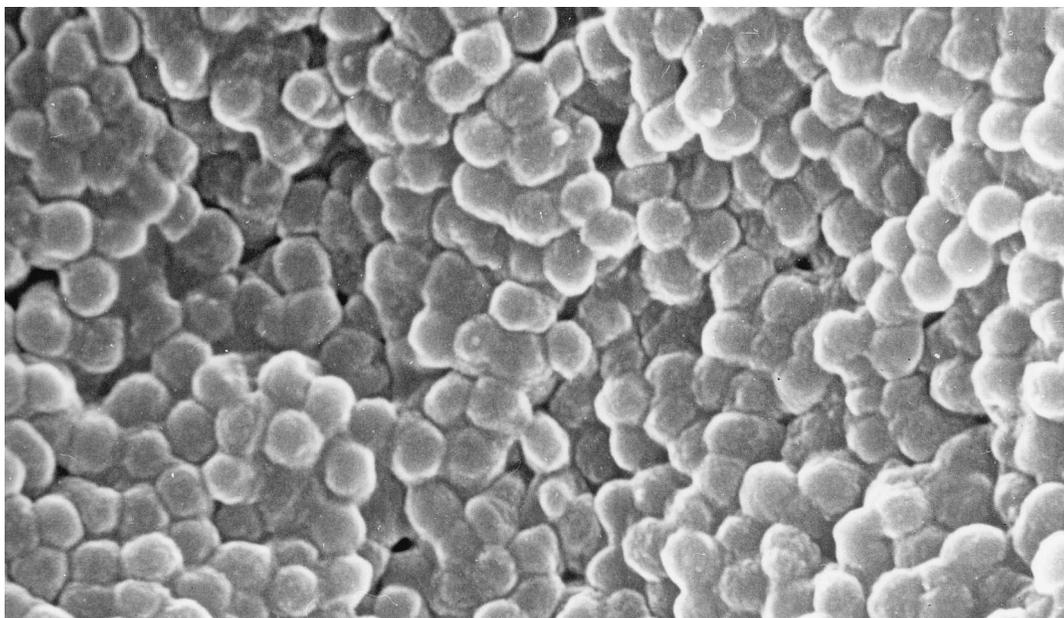


FIG. 1. Scanning electron micrograph of *A. baumannii* type strain ATCC 19606 (final magnification,  $\times 18,000$ ). Prepared and photographed by A. Shelton.

*bacter* infection (7, 29). A novel antibiotic-containing selective medium that combines selectivity with differential characteristics has recently been described (90), and this medium, Leeds Acinetobacter Medium, is useful for the recovery of most *Acinetobacter* spp. from both clinical and environmental sources.

For environmental screening, especially in areas where acinetobacters may be present in only small numbers, liquid enrichment cultivation may also be useful (13). Specimens contaminated with a variety of microorganisms can be used to inoculate a liquid mineral medium containing a single carbon and energy source and ammonium or nitrate salt as the nitrogen source, with a final pH of 5.5 to 6.0. Vigorous shaking during incubation is needed so that any acinetobacters present can outgrow any pseudomonads. After incubation for 24 to 48 h, a loopful of the culture broth is inoculated on to a selective medium and any presumptive *Acinetobacter* colonies are identified further. This method has been used to recover acinetobacters from fecal specimens (79) and from various clinical and environmental specimens (49).

Clinical isolates, which belong mostly to genomic species 2 (*A. baumannii*), 3, or 13TU (Table 1), grow at 37°C or higher, but some other genomic species will grow only at lower temperatures. A general cultivation temperature of 30°C has been recommended (88), but a lower temperature or combination of temperatures may be advisable, depending on the type and origin of the specimen.

#### Morphological, Cultural, and Metabolic Characteristics

Acinetobacters are short, plump, gram-negative (but sometimes difficult to destain) rods, typically 1.0 to 1.5 by 1.5 to 2.5  $\mu\text{m}$  in the logarithmic phase of growth but often becoming more coccoid in the stationary phase (Fig. 1). Pairing or clustering of cells often occurs. Gram stain variability, as well as variations in cell size and arrangement, can often be observed within a single pure culture (14). *Acinetobacter* spp. normally form smooth, sometimes mucoid, pale yellow to greyish-white colonies on solid media, although some environmental strains that produce a diffusible brown pigment have been described

(143). The colonies are comparable in size to those of enterobacteria. All members of the genus are strict aerobes, oxidase negative, catalase positive, and nonfermentative. It is the negative oxidase test that serves as a rapid presumptive test to distinguish *Acinetobacter* spp. from otherwise similar nonfermentative bacteria. Most strains are unable to reduce nitrate to nitrite in the conventional nitrate reduction assay. Some clinical isolates, particularly those belonging to genomic species 4 (*A. haemolyticus*), may show hemolysis on sheep blood agar plates. Although rare *Acinetobacter* strains showing growth factor requirements have been isolated (14, 223), most strains can grow in a simple mineral medium containing a single carbon and energy source. A wide variety of organic compounds can be used as carbon sources, although relatively few strains can use glucose (14), but no single metabolic test enables unambiguous differentiation of this genus from other similar bacteria. Such unambiguous identification relies currently on the ability of extracted DNA to restore the wild-type phenotype to mutant *Acinetobacter* strain BD413 *trpE27* in a transformation assay (104).

#### Species Identification

The division of *Acinetobacter* isolates into genomic species is based on DNA-DNA relatedness. Several different DNA hybridization methods have been used, including a nitrocellulose filter method (92), the S1 endonuclease method (23), the hydroxyapatite method (196), and a quantitative bacterial dot filter method (195). The last method is probably the simplest, but all of these methods are laborious and unsuitable for use in routine microbiology laboratories.

A scheme of 28 phenotypic tests that claimed to discriminate between 11 of the initial 12 genomic species described was originally proposed (23), although only 74 of the 255 strains included in the identification matrix were also allocated to genomic species by DNA-DNA hybridization. Subsequently, a simplified scheme of 16 tests which, except for tests for glucose acidification and detection of hemolysis on sheep blood agar, comprised growth temperature and carbon source utilization

tests was described (24). However, when tested against 198 strains identified by DNA-DNA hybridization (66), only 78% of the strains were identified correctly, with differentiation between the closely related glucose-acidifying genomic species 1, 2, 3, and 13TU (the *A. calcoaceticus*-*A. baumannii* complex) being particularly difficult. A more detailed and successful phenotypic identification scheme has been described (108), but it seems that single or even a few tests cannot be used for unambiguous phenotypic identification of the different genomic species.

As far as commercial identification systems are concerned, the widely used API 20NE system, based largely on carbon source assimilation tests, contained only *A. baumannii*, *A. haemolyticus*, and *A. lwoffii* in the 1993 database release, together with *A. junii* and *A. johnsonii* as a combination, whereas the type species *A. calcoaceticus* and the other genomic species were not included at all. This system sometimes has problems with sensitivity and reproducibility (113), and the differences between the genomic species are so slight that a reliable identification seems unrealistic. Indeed, two studies comparing the API 20NE system with species identification by DNA-DNA hybridization have demonstrated a poor correlation (87, 226). Identification of the clinically relevant genomic species 2 (*A. baumannii*), 3, and 13TU by API 20NE is particularly difficult. Another commercial system, Biolog, differentiates bacteria on the basis of their oxidation of 95 different carbon sources. Promising results with this system have been obtained for 129 *Acinetobacter* strains identified by DNA-DNA hybridization (21), but further refinement of the database is clearly required.

Phenotypic identification methods for individual *Acinetobacter* species are not totally reliable (225), and this can be a source of confusion in clinical microbiology laboratories. To avoid future problems in interpretation of the scientific literature, it is important that persons working with *Acinetobacter* spp. state the precise species identification method used and indicate clearly that identifications are presumptive when this is appropriate.

To avoid the problems with phenotypic species identification, new molecular identification methods are currently being evaluated against DNA-DNA hybridization. Ribotyping studies of strains belonging to the *A. calcoaceticus*-*A. baumannii* complex have shown that this method can generate patterns that are specific for particular genomic species (62). Similarly, restriction analysis of 16S rRNA genes amplified by PCR has indicated that a combination of patterns generated by five restriction enzymes can be used to discriminate most genomic species, including genomic species 1, 2, 3, and 13TU (215), while PCR amplification of 16S–23S spacer regions resolved 15 of 17 genomic species (139).

## NOSOCOMIAL INFECTIONS CAUSED BY *ACINETOBACTER* SPP.

### Overview

Although *A. baumannii* is now recognized to be the *Acinetobacter* genomic species of greatest clinical importance, it is difficult to extrapolate the older literature to say with certainty what a culture reported in 1980 as *A. anitratus* would now be called. Even today, many reports of infection caused by “*A. baumannii*” do not include the necessary tests for specific rather than presumptive identification. Given this qualification, *Acinetobacter* spp. have been isolated from various types of opportunistic infections, including septicemia, pneumonia, endocarditis, meningitis, skin and wound infection, and urinary tract infection (19, 56, 96, 135). The distribution by site of

*Acinetobacter* infection does not differ from that of other nosocomial gram-negative bacteria, with the main sites of infection in several surveys (69, 96, 101, 102) being the lower respiratory tract and the urinary tract. *Acinetobacter* species have emerged as particularly important organisms in ICUs (18, 60, 80, 136, 182, 183, 214), and this is probably related, at least in part, to the increasingly invasive diagnostic and therapeutic procedures used in hospital ICUs over the last two decades.

The true frequency of nosocomial infection caused by *Acinetobacter* spp. is not easy to assess, partly because the isolation of these organisms from clinical specimens may not necessarily reflect infection but, rather, may result from colonization (190). *Acinetobacter* species accounted for 1.4% of all nosocomial infections during a 10-year period (1971 to 1981) in a university hospital in the United States (117), with the principal sites and types of infection including the respiratory tract, bacteremia, peritoneum, urinary tract infection, surgical wounds, meningitis, and skin or eye infection. A more recent study in a university hospital found that hospitalization in an ICU and previous administration of antibiotics were associated with *Acinetobacter* colonization at various body sites in 3.2 to 10.8 per 1,000 patients, with *Acinetobacter* infection accounting for 0.3% of endemic nosocomial infections in critically ill patients and for 1% of nosocomial bacteremia hospital-wide (190). These figures are in agreement with previous observations (33). One study has reported a seasonal incidence of *Acinetobacter* infection (157), with an increase in infection rates in late summer and early winter—a difference that could be related to temperature and humidity changes but one that has not yet been confirmed by other studies.

### Respiratory Infection

Numerous outbreaks of nosocomial pulmonary infection caused by *Acinetobacter* spp. in ICUs have now been described (18, 30–32, 40, 80, 189, 214), and the role played by *Acinetobacter* spp. in ventilator-associated pneumonia appears to be increasing. Regardless of the bacteriological method used to define the cause of pneumonia precisely, several studies have reported that about 3 to 5% of nosocomial pneumonias are caused by *Acinetobacter* spp. (33, 38). In patients with pneumonia enrolled in the National Nosocomial Infection Study (170), this organism accounted for 4% of the total number of pulmonary infections from 1990 to 1992.

Using specific diagnostic techniques, several recent investigators have demonstrated the increasing role played by *Acinetobacter* spp. (probably *A. baumannii*) in nosocomial pneumonia for the subset of ICU patients requiring mechanical ventilation. In studies in which only mechanically ventilated patients were included and bacteriological studies were restricted to uncontaminated specimens obtained by bronchoscopic techniques (54, 197), 15 and 24%, respectively, of all episodes of pneumonia included at least one *Acinetobacter* sp. Although other studies have reported lower infection frequencies of 3 to 5% (33, 38), these data suggest clearly that nosocomial pneumonia caused by *Acinetobacter* spp. is now emerging as a prominent complication of mechanical ventilation. Interestingly, this increased incidence has occurred despite many major advances in the management of ventilator-dependent patients and the routine use of effective procedures to disinfect respiratory equipment.

A number of factors have been suspected or identified as increasing the risk of pneumonia or colonization of the lower respiratory tract by *Acinetobacter* spp.; in the ICU, these include advanced age, chronic lung disease, immunosuppression, surgery, use of antimicrobial agents, presence of invasive de-

vices such as endotracheal and gastric tubes, and type of respiratory equipment (18, 30, 31, 120, 146, 190).

To try to better define the link between the use of ventilators and pneumonia caused by *A. baumannii*, 159 consecutive patients who received mechanical ventilation for >72 h in a medical-surgical ICU over a 13-month period were studied (211). Fiberoptic bronchoscopy with protected-specimen brush and bronchoalveolar lavage was performed on each patient suspected of having pneumonia because of the presence of a new pulmonary infiltrate, fever, and purulent tracheal secretions, but the diagnosis of pulmonary infection was retained only if protected-specimen brush and/or bronchoalveolar lavage specimens grew  $\geq 10^3$  and  $\geq 10^4$  CFU of at least one microorganism per ml, respectively. By the criteria used in this study, nosocomial pneumonia associated directly with *A. baumannii* occurred in 19 (12%) of the 159 study patients, while the organism was present in 27% of ventilator-associated pneumonia cases diagnosed during this period.

Crude mortality rates of 30 to 75% have been reported for nosocomial pneumonia caused by *Acinetobacter* spp., with the highest rates reported in ventilator-dependent patients (18, 54, 197). It is therefore clear that the prognosis associated with this type of infection is considerably worse than that associated with other gram-negative or gram-positive bacteria, with the exception of *P. aeruginosa*. In a study of patients in which the diagnosis was retained only if protected-specimen brush specimens grew  $>10^3$  CFU of at least one organism per ml (54), mortality associated with *Pseudomonas* or *Acinetobacter* pneumonia was >75%, compared with only 55% for pneumonia caused by other organisms ( $P < 0.05$ ).

Although these statistics indicate that nosocomial pneumonia caused by *Acinetobacter* spp. is a severe disease in ventilator-dependent patients, it is difficult to establish whether such critically ill patients would have survived if nosocomial pneumonia had not occurred. In a cohort study in which patients who had developed pneumonia caused by *Acinetobacter* spp. and other organisms were matched carefully with control subjects for the severity of underlying illness and other important variables, such as age, indication for ventilatory support, and duration of exposure to risk (55), the attributable mortality in cases of infection caused by nonfermentative organisms exceeded 40%, with a corresponding relative risk of death of 2.5.

### Bacteremia

Although differentiation between blood specimen contamination by skin inhabitants and true bacteremia is sometimes rather difficult, the most common *Acinetobacter* species causing significant bacteremia is now identified as *A. baumannii* in most series of adult patients in whom proper species identification is made (172). *Acinetobacter* species may be found either as a single pathogen or as part of polymicrobial bacteremia. Immunocompromised patients make up the largest group of adult patients. In these patients, the source of bacteremia is often a respiratory tract infection, with the highest rate of nosocomial bacteremia occurring during the second week of hospitalization. Malignant disease, trauma, and burns seem to be among the most common predisposing factors.

A second important group of patients may consist of neonates. One report from Japan described 19 neonates with *Acinetobacter* septicemia in the neonatal ICU over a period of 30 months. All cases were of late-onset type septicemia in infants hospitalized for long periods, with a mortality rate of 11% (166). The predisposing risk factors for septicemia were low birth weight, previous antibiotic therapy, mechanical ventilation, and the presence of neonatal convulsions. A second re-

port (135) described an outbreak of septicemia in a neonatal ICU which was confined to seven babies receiving parenteral nutrition. All babies had severe clinical symptoms and signs of septic shock, and five required ventilatory support, but they all recovered uneventfully after appropriate antibiotic therapy. In a more recent study from Israel (156), nine cases of *Acinetobacter* sepsis in a neonatal ICU were observed over a period of 31 months. The clinical course was fulminant in four babies who subsequently died. Thus, *Acinetobacter* spp. should be added to the list of organisms capable of causing severe nosocomial infection in neonatal ICUs.

As far as risk factors for adults are concerned, surgical wound infections caused by *Acinetobacter* spp. have been described, and such wound infections may lead to bacteremia. There are also a number of reports in the literature describing *Acinetobacter* bacteremia in burn patients (75, 78). Several studies have shown that there is a correlation between vascular catheterization and *Acinetobacter* infection (158, 177). Changing the catheter insertion site every 48 h and appropriate adherence to aseptic protocols may reduce the risk. An association between *Acinetobacter* bacteremia and the use of transducers for pressure monitoring has been reported (15), and prompt attention to sterilization techniques when handling equipment such as transducers may also reduce the infection rate. In general, the underlying disease seems to determine the prognosis of the patient. The prognosis of patients with malignant disease and burns is rather poor, but trauma patients have a better prognosis. Previous antibiotic treatment is associated with the selection of resistant strains (194).

### Meningitis

Secondary meningitis is the predominant form of *Acinetobacter* meningitis, although sporadic cases of primary meningitis have been reported, particularly following neurosurgical procedures or head trauma (20). Until 1967, there were about 60 reported incidents of *Acinetobacter* meningitis, most of which were community acquired. However, since 1979, the vast majority of cases have been nosocomial infections, with almost all caused probably (but not definitely) by *A. baumannii*. Mortality rates from different series range from 20 to 27%. Most patients have been adult men and had undergone lumbar punctures, myelography, ventriculography, or other neurosurgical procedures, although one patient had posttraumatic otorrhea without intervention (183). A case of *Acinetobacter* meningitis associated with a ventriculoperitoneal shunt with concomitant tunnel infection in which *A. baumannii* was isolated from cerebrospinal fluid has been described (174). Risk factors include the presence of a continuous connection between the ventricles and the external environment, a ventriculostomy, or a cerebrospinal fluid fistula. In addition, the presence of an indwelling ventricular catheter for more than 5 days is an important risk factor. Another important predisposing factor is the heavy use of antimicrobial agents in the neurosurgical ICU. One outbreak subsided spontaneously only when the selective pressure of antibiotics was reduced (183).

A particularly interesting outbreak of *Acinetobacter* meningitis was described in a group of children with leukemia (110) following the administration of intrathecal methotrexate. Of the 20 children who received intrathecal methotrexate, 8 returned within 2 to 19 h of treatment with signs and symptoms of acute meningeal irritation. *Acinetobacter* organisms were isolated from the cerebrospinal fluid of five of these patients, as well as from the methotrexate solution. Three of the children died as a result of meningitis, and five recovered. The

outbreak was caused by the use of inappropriately sterilized needles.

### Urinary Tract Infection

Nosocomial urinary tract infection is caused only infrequently by *Acinetobacter* spp. It occurs most commonly in elderly debilitated patients, in patients confined to ICUs, and in patients with permanent indwelling urinary catheters. Most patients (80%) tend to be men (148), perhaps reflecting the higher prevalence of indwelling urinary catheters in this population as a result of prostatic enlargement. It should, however, be noted that not every isolation of *Acinetobacter* spp. from the urinary tract of patients with an indwelling urinary catheter can be correlated with actual infection (84).

### Other Miscellaneous Infections

A few very rare cases of native-valve infective endocarditis caused by *Acinetobacter* spp. have been reported (76). Dental procedures and open heart surgery have been identified as possible inciting events. *Acinetobacter* infective endocarditis does not differ clinically from infective endocarditis caused by other microorganisms, but there are wide variations in the presentation and clinical course of the disease.

*Acinetobacter* spp. can also cause peritonitis in patients undergoing continuous ambulatory peritoneal dialysis. It is difficult to be certain that all such episodes are nosocomial, but technique failure and diabetes mellitus are the main underlying risk factors. The mean duration of risk factors before the onset of peritonitis ranged from 2 to 13 months. The most common manifestations were abdominal pain or cloudy dialysate, but only a minority of patients had fever. Most patients respond to antibiotic therapy without the need to interrupt continuous ambulatory peritoneal dialysis (57, 121, 213).

*Acinetobacter* cholangitis and septic complications following percutaneous transhepatic cholangiogram and percutaneous biliary drainage have been reported, primarily among elderly patients with obstructive jaundice caused by malignant disease or choledocholithiasis. In one study, 13.5% of patients undergoing transhepatic cholangiography or biliary drainage developed infection, with the most common isolates being *Enterobacter cloacae* and *Acinetobacter* spp. (165). Other, more rare case reports include typhlitis after autologous bone marrow transplantation (133) and osteomyelitis and extremity infections following injury (44, 125). Eye infections following trauma have been described (123, 126). Penetrating keratoplasty (228) and the fitting of contact lenses (11) have also caused ophthalmic infections with *Acinetobacter* spp.

## PATHOGENESIS OF *ACINETOBACTER* INFECTIONS

### Predisposing Factors

Various risk factors predisposing to severe infection with *Acinetobacter* spp. have been identified; some of these also apply to organisms causing other nosocomial infections. Susceptible patients include those who have recently undergone major surgery, those with severe underlying disease (e.g., malignancy, burns or immunosuppression), and particularly the elderly, although outbreaks of *Acinetobacter* infection have also been described in neonates (135, 189).

Most detailed studies of risk factors have involved studies of respiratory tract infections, and a number of factors have been suspected or identified as increasing the risk of pneumonia or colonization of the lower respiratory tract by *Acinetobacter* spp. (probably *A. baumannii* in most cases) in the ICU; these in-

clude advanced age, chronic lung disease, immunosuppression, surgery, use of antimicrobial agents, presence of invasive devices such as endotracheal and gastric tubes, and type of respiratory equipment (18, 30, 31, 120, 146, 190). Although many of these variables are likely to be interrelated, only a few studies of potential risk factors have used appropriate statistical modelling to define independent risk determinants (206).

A case-control analysis in which ICU patients with respiratory colonization or infection with *Acinetobacter* spp. were compared with matched ICU controls who had other gram-negative bacilli in their sputum (146) was used to investigate an outbreak of infection with aminoglycoside-resistant *Acinetobacter* spp. involving 98 patients at a university hospital in North Carolina. The duration of the ICU stay before colonization or infection with *Acinetobacter* spp. was significantly longer for infected patients than for controls (14.7 versus 5.9 days;  $P = 0.002$ ). Although exposure to invasive devices and procedures did not differ significantly between the two groups, infected patients received respiratory therapy for significantly longer than did controls (14.7 versus 6.6 days;  $P = 0.006$ ). Aminoglycoside usage in the two groups was comparable, but infected patients received an aminoglycoside for a longer duration before colonization or infection than did controls (9.0 versus 6.1 days;  $P = 0.08$ ) and had also received more cephalosporins than did controls (1.9 versus 1.2;  $P = 0.018$ ).

A similar study of 40 patients infected or colonized with *A. baumannii* (120), in which these patients were compared by logistic regression analysis with 348 noninfected and noncolonized patients who were present in the ICU at the same time, also demonstrated that the severity of the underlying disease, as evaluated by the APACHE II score (112), and the presence of a previous infection that required antimicrobial treatment were independent factors for acquiring *A. baumannii* infection.

In conclusion, extended ICU care as a result of severe underlying disease, prolonged respiratory therapy with mechanical ventilation, and previous antimicrobial therapy are all key factors in predisposing to *Acinetobacter* infection. Since the only factor amenable to control in the ICU setting is antimicrobial therapy, avoidance of unnecessary antibiotics should be a high priority in management of such patients. The use of antibiotics probably alters the normal flora and results in the selection of resistant microorganisms such as *Acinetobacter* spp.

### Virulence of *Acinetobacter* spp.

Although *Acinetobacter* spp. are considered to be relatively low-grade pathogens (105, 147, 185), certain characteristics of these organisms may enhance the virulence of strains involved in infections; these characteristics include (i) the presence of a polysaccharide capsule formed of L-rhamnose, D-glucose, D-glucuronic acid, and D-mannose (109), which probably renders the surface of strains more hydrophilic, although hydrophobicity may be higher in *Acinetobacter* strains isolated from catheters or tracheal devices (109, 159); (ii) the property of adhesion to human epithelial cells in the presence of fimbriae and/or capsular polysaccharide (152, 159, 160); (iii) the production of enzymes which may damage tissue lipids (153); and (iv) the potentially toxic role of the lipopolysaccharide component of the cell wall and the presence of lipid A (10). In common with other gram-negative bacteria, *Acinetobacter* spp. produce a lipopolysaccharide responsible for lethal toxicity in mice, pyrogenicity in rabbits, and a positive reaction in the *Limulus* amoebocyte lysate test. The production of endotoxin in vivo is probably responsible for the disease symptoms observed during *Acinetobacter* septicemia.

*A. baumannii* appears to have only limited virulence in mice (50% lethal dose,  $10^6$  to  $10^8$  CFU per mouse) when inoculated intraperitoneally, even in neutropenic mice. However, experimental studies in a murine model of *Acinetobacter* pneumonia have shown that this pneumonia closely resembles that in humans (103). Experimentally, mixed infections combining other bacteria with *Acinetobacter* spp. are more virulent than infections with *Acinetobacter* spp. alone (140). Slime produced by the *Acinetobacter* strain studied was considered to be the main factor responsible for the enhancement of virulence in mixed infections, but few acinetobacters are slime producing; indeed, of 100 isolates tested (140), only 14 had slime producing ability. The same study demonstrated that slime was associated with cytotoxicity against neutrophils and inhibition of the migration of neutrophils into peritoneal exudate of mice. No correlation was observed between the amount of slime produced and the degree of virulence.

The ability of a bacterium to obtain the necessary iron for growth in the human body is also an important virulence determinant, and some *Acinetobacter* strains have been shown to produce siderophores, such as aerobactin, and iron-repressible outer membrane receptor proteins (2, 51, 186).

## EPIDEMIOLOGY

### Human Carriage

Acinetobacters can form part of the bacterial flora of the skin, particularly in moist regions such as the axillae, groin, and toe webs, and it has been suggested that at least 25% of normal individuals carry *Acinetobacter* spp. on their skin (188, 192). *Acinetobacter* spp. have also been found occasionally in the oral cavity and respiratory tract of healthy adults (69, 161), but the carriage rate of *Acinetobacter* spp. in nonhospitalized patients, apart from on the skin, is normally low.

In contrast, the carriage rate may be much higher in hospitalized patients, especially during outbreaks of infection. Throat swabs have been found to be positive for *Acinetobacter* spp. in 7 to 18% of patients, while tracheostomy swabs were positive in 45% of hospitalized patients (162). High colonization rates of the skin, throat, respiratory system, or digestive tract, of various degrees of importance, have been documented in several outbreaks. In particular, outbreaks involving mechanically ventilated ICU patients are associated with a high colonization rate of the respiratory tract (7, 30, 60, 146), which may indicate contamination of respiratory therapy equipment as the possible source of an outbreak. In addition, patients often have skin colonization during outbreaks. Such colonization of patients plays an important role in subsequent contamination of the hands of hospital staff during trivial contacts, thereby contributing to the spread and persistence of outbreaks (67). Colonization of the digestive tract of patients with *Acinetobacter* spp. is unusual (79), but several studies have documented oropharyngeal colonization of patients with respiratory tract colonization, and digestive tract colonization has been reported to be a major reservoir of resistant strains (166, 227).

Several conclusions regarding colonization in hospitalized patients can be drawn from the published studies: (i) a high rate of colonization can be found in debilitated hospitalized patients, especially during outbreak situations; (ii) a predominant site of colonization is the skin, but other sites, such as the respiratory or digestive tract, may also be involved and may predominate on certain occasions; and (iii) the observed discrepancies between carriage rates for outpatients and hospitalized patients suggests that infecting or colonizing organisms in hospital-acquired infections may derive more often from

cross-transmission or hospital environmental sources rather than from endogenous sources in patients.

The large proportion of colonized patients in a given hospital setting means that the differentiation between colonization and infection may not be straightforward. Nosocomial *Acinetobacter* infections may involve any site, but they predominate in the respiratory tract, urinary tract, and wounds. Many isolates from the skin and the respiratory tract should still be considered to be colonizing rather than infecting organisms. A steady increase (from 25 to 45%) in the proportion of *Acinetobacter* isolates from superficial wounds has been recorded over the past decade (94), and the skin, respiratory tract, and superficial wounds should therefore be considered to be potential important reservoirs of infecting organisms during outbreak situations.

### Persistence in the Hospital Environment

Numerous studies have documented the presence of *Acinetobacter* spp. in the hospital environment, but rates of positive cultures may vary widely, depending on the epidemiological setting. *Acinetobacter* spp. have been found in 27% of hospital sink traps and 20% of hospital floor swab cultures (162). Air contamination in the absence of a colonized patient is comparatively rare, but several studies have documented extensive contamination by *Acinetobacter* spp. of the environment, including respirators and air samples, in the vicinity of infected or colonized patients (40). During an outbreak of infection originating from an ICU, 12 (11.5%) of 104 air samples from wards harboring colonized patients were positive for *Acinetobacter* spp., as well as 7 (8%) of 89 sink trap swabs and 13 (17%) of 75 samples from bedside cupboards in the same areas (39). Extensive contamination of the environment, including air samples, was found in an outbreak of infection with multiresistant *Acinetobacter* spp. (7); again, contamination was found essentially in the vicinity of infected or colonized patients, with 16 of 82 settle plate cultures from the environment of three colonized patients found to be positive. Bed linen from colonized patients was positive consistently, but linen from noncolonized patients was also positive on several occasions, as well as overblankets from empty beds in the vicinity of one ventilated colonized patient and bed curtains around colonized patients. Persistent environmental contamination was documented for up to 13 days after the discharge of a patient. A further notable example of the role of the immediate bed environment in the dissemination of *Acinetobacter* spp. was seen in a large outbreak involving 63 of 103 patients admitted to a burns unit over a 21-month period (182). The outbreak was traced to contamination of mattresses through breaches in plastic covers that allowed water penetration and persistence of the organism in the wet foam of the mattresses. More recently, feather pillows were found to be contaminated with considerable numbers of acinetobacters in an outbreak of infection in The Netherlands (226). It is therefore apparent that contaminated bedding materials may play an important role in the nosocomial dissemination of these organisms.

The above data indicate that certain *Acinetobacter* spp. can persist in the environment for several days, even in dry conditions on particles and dust, thereby probably contributing to the development and persistence of outbreaks. It has been reported that *Acinetobacter* cells can survive on dry surfaces for durations even longer than that found for *Staphylococcus aureus* (67, 132). Environmental contamination during an outbreak in a pediatric ICU was demonstrated on various equipment and surfaces in the unit (telephone handles, door pushplates, patient charts, tabletops, etc.), all of which were

probably contaminated by the hands of staff (67). Similarly, an epidemic strain of multiresistant *Acinetobacter* spp. has been shown to survive for up to 6 days after inoculation on to dry filter paper, a duration similar to that found with *S. aureus*, which persisted for 7 days, but significantly greater than the survival times for *Escherichia coli* and *Pseudomonas* spp., both of which persisted for 24 h or less (7). Prolonged survival of *Acinetobacter* spp. on hospital floors and air-dried washcloths has also been described (30). Survival is probably also helped by the ability of *Acinetobacter* spp. to grow at a range of different temperatures and pH values (13, 14, 88, 201).

In summary, *Acinetobacter* spp. have unique characteristics among nosocomial gram-negative bacteria that favor their persistence in the hospital environment. These organisms spread easily in the environment of infected or colonized patients and can persist in that environment for many days, a factor that may explain their propensity for causing extended outbreaks. However, it should be noted that acinetobacters are ubiquitous organisms that can also be isolated readily from nonclinical sources such as soil, drinking and surface waters, sewage, and a variety of different foodstuffs (201). There appears to be a significant population difference between the genomic species found in clinical specimens and those found in other environments (59), and it is therefore vital that acinetobacters be identified to the genomic species level and then typed before epidemiological conclusions can be drawn.

The dissemination and persistence of *Acinetobacter* spp. in the hospital environment probably accounts for the specific role that has been reported for contaminated materials as a reservoir of infection, especially during outbreaks. In some persistent outbreaks, contaminated materials have been shown to act as a source of the outbreak. Materials used for respiratory therapy or support have been implicated in many such cases (32, 80, 187). Thus, an outbreak of 24 cases of infection with *Acinetobacter* spp., occurring mostly in debilitated patients with intravascular catheters, was traced to contaminated room air humidifiers (187). Contaminated air samples were found up to 10 m from the humidifiers, and probable skin colonization of patients in the vicinity of the devices resulted in intravascular catheter infection. A similar outbreak occurred in patients undergoing peritoneal dialysis (1), and contamination of dialysis fluid bottles, via a contaminated water bath used to warm the dialysis fluid, was demonstrated. This outbreak was controlled after revision of the decontamination procedures for the heating buckets and for starting infusion of dialysis fluids. Other outbreaks have involved inadequate sterilization of autoclavable reusable needles used for administration of intrathecal methotrexate in patients with leukemia (110), defective heating of a washing machine used for decontamination of reusable ventilator tubings (32), and inadequate decontamination of respiratory monitoring devices and resuscitation bags (80, 189, 214). Ethylene oxide sterilization of such contaminated equipment has been shown to be effective. Radiation resistance of *Acinetobacter* clinical isolates has been demonstrated (36), and these results indicate that special attention should be paid to medical devices that are normally sterilized by irradiation, particularly devices used in ICUs.

However, although respiratory equipment may be responsible for persistent outbreaks as a result of inadequate decontamination between use in consecutive patients, such equipment may act in some instances only as an intermediate reservoir of organisms and not as the primary source of infection. Thus, in one outbreak, a respiratory therapist with hand lesions from dermatitis was found to be a chronic carrier of *Acinetobacter* spp. and was contaminating the equipment during assembly and testing (30). Medical equipment may there-

fore become contaminated both by the patients themselves and by staff during handling, and the latter possibility should always be considered when outbreaks of infection occur, especially in respiratory ICUs.

## TYPING SYSTEMS

Typing methods are important tools for establishing the sources and mode(s) of transmission for epidemic strains. Early attempts to develop typing systems for *Acinetobacter* spp. have been reviewed previously (22). No single typing system has so far gained acceptance for typing *Acinetobacter* spp., and this area is still the subject of research. The following sections describe the different typing systems that are currently being applied to *Acinetobacter* spp. Some of these are based on the latest taxonomic developments, while others aim simply to discriminate individual strains without determining their precise genomic species. The inherent advantages and disadvantages of the different approaches to bacterial typing have been considered elsewhere (203).

### Biotyping

Biochemical profiles comprising binary characteristics (with results being scored as positive or negative) can be used for comparative typing of strains. A biotyping system consisting of five tests (24, 26), devised originally for dividing isolates of *A. baumannii* into 19 biotypes, has also been used to type the related genomic species 3 and 13TU in various hospital outbreaks of infection (26, 64, 65). The API 20NE system has been used to distinguish 31 different biotypes among 122 different *Acinetobacter* strains (202), but this system sometimes has problems with sensitivity and reproducibility (113). However, cluster analysis of carbon source growth assays has been used to identify a major grouping of isolates that was related to the epidemiological origin of the strains (48). Other commercial systems with large numbers of substrates may therefore be of use, but such systems have yet to be assessed with epidemiologically defined strains of *Acinetobacter* spp. from different outbreaks.

### Antibiograms

Numerous studies have used antibiotic susceptibility patterns (antibiograms) to detect emerging resistance patterns and to group similar isolates (5, 7, 17, 100, 190, 219), often on the basis of MICs, breakpoints, or zone diffusion sizes. Such results are often expressed as resistant, susceptible, or intermediate. A more informative approach uses the actual diameters of inhibition zones in disc diffusion tests for cluster analysis, and such groupings have been shown to correlate well with other typing and epidemiological data (45). However, it must be emphasized that antibiogram typing results should be interpreted with caution, since unrelated strains may exhibit the same antibiogram (100) and changes in susceptibility may occur during episodes of infection.

### Serotyping

There have been numerous attempts to type *Acinetobacter* strains by serological reactions, but only limited success was obtained in early work (3, 41, 81), and most such schemes have been rendered obsolete by the taxonomic developments in the genus. More recent work involving checkerboard tube agglutinations and reciprocal cross-absorptions with polyclonal rabbit immune sera against heated cells has allowed the delineation of 34 serovars in *A. baumannii* and 26 serovars in genomic

species 3 (207–209). However, antigenic differences between *A. baumannii* and genomic species 3 serovars were not entirely satisfactory, and the relationship with strains belonging to genomic species 13TU is unclear. Further work with large numbers of epidemiologically defined strains identified unambiguously by DNA-DNA hybridization is required to test the utility of this method.

### Phage Typing

Two complementary sets of bacteriophages (comprising 25 phages, allowing the identification of 125 phage types, and 14 phages, allowing the identification of 25 phage types) have been used in a number of different epidemiological studies of *Acinetobacter* isolates from France and other European countries (26, 29, 68, 99, 167, 218). Predominant phage types (numbers 17 and 124) have been identified in some outbreaks (167, 218). However, this system has been used only at the Phage Typing Center of the Institut Pasteur in Paris, and it seems that a substantial proportion of strains from other geographical locations may be nontypeable. Although some doubt has been cast upon the reproducibility of the results (26), phage typing may be useful, albeit time-consuming, when used in conjunction with other typing methods.

### Bacteriocin Typing

Two reports of bacteriocin typing of *Acinetobacter* isolates have been published. In the first study (9), 176 strains were typed by means of 10 indicator strains that were susceptible to bacteriocins. Overall typeability was 65%, but 56% of strains belonged to only two groups. The second study (189) used 19 bacteriocin-containing lysates to type 100 strains. Only 46% of strains were typeable, but 16 isolates, including 11 from an outbreak, belonged to the same type. Bacteriocin typing of isolates identified by DNA-DNA hybridization has not been reported, and the usefulness of this method for typing clinically important genomic species remains to be investigated.

### Protein Profiles

Both cell envelope and whole-cell protein patterns have been used in a series of epidemiological and taxonomic studies of *Acinetobacter* spp. Analysis of cell envelope protein patterns by sodium dodecyl sulfate-polyacrylamide gel electrophoresis has shown heterogeneity in unrelated strains, while multiple isolates from patients or outbreaks were indistinguishable (46, 49). This method has been used successfully to trace specific strains during endemic episodes and outbreaks in hospitals (39, 47, 226). Electrophoretic analysis of whole-cell protein fractions has the advantage that sample preparation is simpler than preparation of cell envelopes. Similarities between *Acinetobacter* isolates from outbreaks and dissimilarities in unrelated control strains have been reported in several studies (4, 5, 128), but as with other phenotypically based methods, apparent differences between isolates from a common origin should be interpreted with caution.

### Multilocus Enzyme Electrophoretic Typing

Multilocus enzyme electrophoresis investigates the relative electrophoretic mobilities of a large number of cellular enzymes (178). Investigation of 27 esterases and 2 dehydrogenases in 81 *Acinetobacter* isolates identified between 2 and 17 variants for each enzyme (150). A separate study of 13 enzymes in 65 *Acinetobacter* clinical isolates identified 14 different types, of which 1 type was found in 41 multiresistant isolates with

common whole-cell protein profiles (193). These results suggest that multilocus enzyme electrophoresis has the potential to be developed into a useful technique for strain identification.

### Plasmid Profiles

Numerous studies have now used plasmid typing as a rapid and simple method for identifying *Acinetobacter* strains (5, 61, 65, 67, 91, 113, 175). The plasmids found in *Acinetobacter* strains vary considerably in both size and number. Thus, between one and four plasmids varying in size from 2.1 to >100 kb were found in 13 strains belonging to the *A. calcoaceticus*-*A. baumannii* complex, but no plasmids at all were found in 10 other strains examined (65). Nevertheless, analysis of plasmid profiles has been useful in delineating several outbreaks of *Acinetobacter* infection (15, 80, 113, 219). Strains with similar plasmid profiles have been differentiated further either by restriction endonuclease digestion of plasmid DNA to generate plasmid fingerprints (113, 219) or by hybridization of plasmids with a labelled probe from one of the strains (65). Overall, it can be concluded that plasmid typing may be extremely useful in epidemiological studies, provided that the results are interpreted in conjunction with other typing methods and the origin of the strains is known.

### Analysis by Pulsed-Field Gel Electrophoresis

Analysis by pulsed-field gel electrophoresis of restriction fragment length polymorphisms generated from intact chromosomal DNA has been used to compare fingerprints obtained from *Acinetobacter* strains following restriction with *ApaI* (77, 176, 191), *SmaI* (6), *ApaI* and *SmaI* (74), and *NheI* and *SmaI* (190). These studies have indicated considerable DNA polymorphism in the clinically important genomic species 2 (*A. baumannii*), even within biotypes, and good correlation between strains from within defined outbreaks or multiple isolates from single patients. Equipment for pulsed-field gel electrophoresis is costly, while the preparation of intact chromosomal DNA and subsequent digestion and electrophoresis require several days. Nevertheless, pulsed-field gel electrophoresis seems to provide highly discriminatory results and extremely useful epidemiological information.

### Ribotyping

This method has been described in detail for *Acinetobacter* spp. (62). Briefly, purified chromosomal DNA is digested with restriction enzymes, electrophoresed, blotted, and then hybridized with a labelled cDNA probe derived from *E. coli* rRNA. Patterns generated by restriction with *EcoRI*, *ClaI*, or *SalI* have been used to investigate 70 strains that had been identified as either *A. calcoaceticus*, *A. baumannii*, or genomic species 2 or 13TU by DNA-DNA hybridization (62). Excellent reproducibility was observed, and combined use of the three enzymes generated 52 different types among the 70 unrelated strains studied. Ribotype patterns within outbreaks have been shown to be stable and to correlate with results obtained by other typing methods (45). However, although the diversity of ribotypes within the *A. calcoaceticus*-*A. baumannii* complex is considerable, common patterns in apparently unrelated strains have also been observed (45, 62). Again, this is a laborious technique, but it can provide valuable epidemiological information, particularly when used in combination with other typing methods.

### PCR-Based Methods

Fingerprinting on the basis of PCR amplification of DNA sequences by either specific or random primers is being used increasingly for typing microorganisms. The core region of bacteriophage M13 has been used as a single primer to determine the relatedness of *A. baumannii* strains (77). The repetitive elements ERIC1 and ERIC2 (217) were also used successfully as single primers in one study for typing *A. baumannii* isolates from a tertiary-care hospital (190). In contrast, ERIC1 and ERIC2 primers were not useful for typing the isolates of *A. baumannii* from another outbreak (155), whereas the repetitive elements REP1-RI plus REP2-I generated PCR fingerprints that discriminated between epidemic and sporadic strains of *A. baumannii* and demonstrated four discrete clusters that were unique epidemiologically. Further studies are required to assess the longitudinal reproducibility of PCR-based typing methods. Comparisons between typing results obtained in different centers may be difficult, but these methods are extremely rapid and seem to be useful for tracing epidemic strains during outbreaks on a day-to-day basis.

### CLINICAL ANTIBIOTIC RESISTANCE

Numerous reports in the medical and scientific literature have documented the high rates of antibiotic resistance found in *Acinetobacter* spp. (17, 29, 56, 101, 117, 190). A particular concern has been the frequent multiple antibiotic resistance exhibited by nosocomial acinetobacters and the resulting therapeutic problems involved in treating patients with nosocomial infections in ICUs. Until the early 1970s, nosocomial *Acinetobacter* infections could be treated successfully with gentamicin, minocycline, nalidixic acid, ampicillin, or carbenicillin, either as single agents or in antibiotic combinations, but increasing rates of resistance began to be noticed between 1971 and 1974. Since 1975, successive surveys have shown increasing resistance in clinical isolates of *Acinetobacter* spp. (58, 71, 93, 141). High proportions of strains have become resistant to older antibiotics; indeed, many acinetobacters are now resistant to clinically achievable levels of most commonly used antibacterial drugs, including aminopenicillins, ureidopenicillins, narrow-spectrum (cephalothin) and expanded-spectrum (cefamandole) cephalosporins (93, 127), cephamycins such as cefoxitin (58), most aminoglycosides-aminocyclitols (43, 50, 72, 93), chloramphenicol, and tetracyclines. For some relatively new antibiotics, such as broad-spectrum cephalosporins (cefotaxime, ceftazidime), imipenem, tobramycin, amikacin, and fluoroquinolones, partial susceptibility remains, but the MICs of these antibiotics for *Acinetobacter* isolates have increased substantially in the last decade. Imipenem remains the most active drug; indeed, until recently, imipenem retained activity against 100% of strains (8, 129, 177, 220), and in some reports the only active drugs were imipenem and the polymyxins. Unfortunately, the most recent extensive analyses of hospital outbreaks have documented the spread of imipenem-resistant strains (70, 191). This is a particularly worrying development which threatens the continued successful treatment of *Acinetobacter* infections. Most resistance to imipenem has been observed in strains identified as *A. baumannii*, while the MIC of carbapenems for non-*A. baumannii* strains has remained below 0.3 mg/liter, but the widespread emergence and/or spread of resistance to imipenem is likely to pose a serious threat in the near future.

Differences in antibiotic susceptibility have been observed between countries, probably as a result of environmental factors and different patterns of antimicrobial usage. Thus, most

studies report 50 to 80% of isolates to be insusceptible to gentamicin and tobramycin, while aminoglycosides no longer seem to be active at clinically achievable levels against *A. baumannii* isolates from Germany (173). Similarly, most *Acinetobacter* isolates in France, which were originally susceptible to fluoroquinolones, became insusceptible (75 to 80%) to pefloxacin and other fluoroquinolones within 5 years of the introduction of these antibiotics.

Species other than *A. baumannii* isolated from the hospital environment, i.e., *A. lwoffii*, *A. johnsonii*, and *A. junii*, are involved less frequently in nosocomial infection and are generally more susceptible to antibiotics (60, 101, 210). Strains of *A. lwoffii* are more susceptible to  $\beta$ -lactams than is *A. baumannii*. *A. haemolyticus* isolates are normally insusceptible to aminoglycosides and rifampin, but rifampin has mean MICs for *A. baumannii* of 2 to 4 mg/liter and has been used effectively in synergic combination with imipenem in ICUs in France (17).

## BIOCHEMICAL AND GENETIC MECHANISMS OF ANTIBIOTIC RESISTANCE

### Genetics of Resistance

*Acinetobacter* is a genus that appears to have a propensity to develop antibiotic resistance extremely rapidly, perhaps as a consequence of its long-term evolutionary exposure to antibiotic-producing organisms in a soil environment. This is in contrast to more "traditional" clinical bacteria, which seem to require more time to acquire highly effective resistance mechanisms in response to the introduction of modern radical therapeutic strategies; indeed, it may be their ability to respond rapidly to challenge with antibiotics, coupled with widespread use of antibiotics in the hospital environment, that is responsible for the recent success of *Acinetobacter* spp. as nosocomial pathogens. However, although all three of the major modes of chromosomal gene transfer have been demonstrated in *Acinetobacter* spp. (82, 107, 204), only conjugation has so far been shown to play a significant role in the transfer of antibiotic resistance genes between members of this genus (35, 205).

Plasmids and transposons play an important role in the biology of most prokaryotic organisms, and *Acinetobacter* spp. appear to be no exception to this generalization (200). Several studies have reported that >80% of *Acinetobacter* isolates carry multiple indigenous plasmids of variable molecular size (61, 175), although other workers report problems in isolating plasmid DNA from *Acinetobacter* spp., often because of unappreciated difficulties in lysing the cell wall of these organisms. Although many clinical isolates of *A. baumannii* show widespread and increasing resistance to a whole range of antibiotics, there have been only a few studies (see below) in which plasmid-mediated transfer of resistance genes has been demonstrated. However, as postulated by Towner (200), failure to observe transfer of resistance may simply reflect the absence of a suitable test system for detecting such transfer. For historical reasons, workers attempting to transfer plasmids from clinical isolates of any gram-negative species have tended to use *E. coli* K-12 as a recipient strain. Complex and varied transfer frequencies of standard plasmids belonging to different incompatibility groups have been observed between *Acinetobacter* strain EBF 65/65 and *E. coli* K-12, and a number of these plasmids required an additional mobilizing plasmid for retransfer to occur (35). Accordingly, it is not surprising that most reported cases of indigenous transmissible antibiotic resistance from *Acinetobacter* spp. have been associated with plasmids belonging to broad-host-range incompatibility groups (200). There remains a need to rigorously assess the potential for

TABLE 3.  $\beta$ -Lactamases described in *Acinetobacter* spp.

Enzyme or strain	Location of gene	Predominant substrate	Molecular size (kDa)	Reference
TEM-1	Plasmid	Penicillin	29	149
TEM-2	Plasmid	Penicillin	29	43
CARB-5	Plasmid	Penicillin	29	12
ARI-1	Plasmid	Carbapenem	23	144
NCTC7844	Chromosome	Cephalosporin	30	127
ML4961	Chromosome	Cephalosporin	38	83
ACE-1	Chromosome	Cephalosporin	~500	86
ACE-2	Chromosome	Cephalosporin	60.5	86
ACE-3	Chromosome	Cephalosporin	32.5	86
ACE-4	Chromosome	Cephalosporin	>1,000	86
SHV-like	Unknown	Penicillin	UK <sup>a</sup>	95
SHV-like	Unknown	Penicillin	UK	220

<sup>a</sup> UK, unknown.

mobilization of antibiotic resistance genes to and from the different genomic *Acinetobacter* species that have now been delineated. Transposons probably play an important role, in conjunction with integrons (37), in ensuring that particular novel genes can become established in a new gene pool, even if the plasmid vectors that transferred them are unstable, and there have been several reports of chromosomally located transposons carrying multiple antibiotic resistance genes in clinical isolates of *Acinetobacter* spp. (200). However, studies aimed at defining the gross topological structure and organization of bacterial genetic material are currently somewhat unfashionable, and, as far as the genus *Acinetobacter* is concerned, there have been few significant advances since it was demonstrated that strain EBF 65/65 had a circular chromosomal linkage map (198). A total of 29 genetic loci have been mapped on the chromosome of strain EBF65/65 (222), but only one study has examined possible transposon insertion sites in the chromosome of this genus (199).

The following sections summarize the known biochemical and genetic mechanisms of resistance of *Acinetobacter* spp. to the major groups of antibiotics.

### $\beta$ -Lactams

As with other gram-negative organisms, most resistance to  $\beta$ -lactams in *Acinetobacter* spp. is associated with the production of  $\beta$ -lactamases, including the widely distributed TEM-1 and TEM-2 enzymes (43, 72, 98, 149). An analysis of 76 ticarcillin-resistant (MIC, >256 mg/liter) *Acinetobacter* strains for their  $\beta$ -lactamase content found penicillinase activity in only 41% of the resistant strains (98), of which the majority produced an enzyme with a pI of 5.4 (TEM-1 like), although a few had an enzyme with a pI of 6.3, which is characteristic of the  $\beta$ -lactamase CARB-5. Some  $\beta$ -lactamase activity was also identified with a pI above 8.0. These were undefined enzymes that were presumed to be chromosomally encoded cephalosporinases because of their high pI. A separate study identified cephalosporinase activity in 98% of the clinical isolates of *A. baumannii* studied (220), and it therefore seems that cephalosporinases are the predominant  $\beta$ -lactamases in this species.

Four such enzymes, designated ACE-1 to ACE-4, have been studied in detail (86). All four enzymes were identified as cephalosporinases, although some possessed a little activity against penicillins and none had detectable hydrolyzing activity against aztreonam or the broad-spectrum cephalosporins, ceftazidime or cefotaxime. All four enzymes showed their maximum activity against cephaloridine and, except for ACE-4, showed good activity against cephradine. ACE-1 showed the

broadest spectrum of activity with some hydrolysis of cefuroxime. The contribution of these chromosomal  $\beta$ -lactamases appears to be important in the expression of  $\beta$ -lactam resistance but may work in concert with a permeability reduction and altered penicillin-binding proteins that may already confer some inherent resistance (142, 168). The acquisition of plasmid-encoded penicillinases does not seem to have been of paramount importance in the long-term  $\beta$ -lactam resistance of this genus. There has so far been only one suggestion (95) of a possible plasmid-encoded extended-spectrum  $\beta$ -lactamase in *Acinetobacter* spp.

A particularly worrying development is the identification of a novel  $\beta$ -lactamase, designated ARI-1, in an imipenem-resistant strain of *A. baumannii* isolated from a blood culture at the Royal Infirmary, Edinburgh, in 1985 (144). This enzyme hydrolyzes both imipenem and azlocillin but not cefuroxime, ceftazidime, or cefotaxime. Direct conjugative transfer of the ARI-1 gene from its original *A. baumannii* host to an *A. junii* recipient has been demonstrated (169), and the same plasmid can be visualized in the donor and recipient strains. These last observations suggest strongly that ARI-1 is a plasmid-encoded carbapenemase, a development that may have extremely serious long-term consequences.

The  $\beta$ -lactamases known to exist in *Acinetobacter* spp. are summarized in Table 3.

### Aminoglycosides

Aminoglycosides are used widely for the treatment of *Acinetobacter* infections, and increasing numbers of highly resistant strains have been reported since the late 1970s. All three types of aminoglycoside-modifying enzymes have been identified within clinical *Acinetobacter* strains (Table 4), but geographic variations in the incidence of particular genes has been observed; e.g., the gene for AAC(3)-Ia was found frequently in *Acinetobacter* strains from Belgium (36 of 45 strains) but was observed less frequently in strains from the United States (3 of 17 strains) and not at all in strains from Argentina (180, 181). In addition, some strains have been observed to contain more than one aminoglycoside resistance gene, with as many as six different resistance genes being identified in some isolates. It

TABLE 4. Aminoglycoside-modifying enzymes identified in *Acinetobacter* spp.

Enzyme	Reference(s)
<b>Acetyllating</b>	
AAC(6')	114, 116, 130, 179
AAC(2')I	50
AAC(3)I	16, 220
AAC(3)II	131
AAC(3)V <sup>a</sup>	53, 181
AAC(3)IV	181
<b>Adenylating</b>	
ANT(3'')I	179
AAD(3'')(9) <sup>a</sup>	43, 72, 131, 220
ANT(2'')I	131
AAD(2'') <sup>a</sup>	53, 181
<b>Phosphoryllating</b>	
APH(3')I	16, 43, 72, 181
APH(3')II	130
APH(3')III	97, 130
APH(3')VI	114, 115, 124, 181, 220
APH(3')I	52

<sup>a</sup> Enzymatic activity detectable only in vitro.

has been suggested that the novel gene *aac(6')-I<sub>g</sub>*, identified only in *A. haemolyticus*, where it is responsible for amikacin resistance, may be used to facilitate the identification of this species (116).

Few published studies have been devoted to investigating the genetic nature of aminoglycoside resistance in *Acinetobacter* spp., but both plasmid and transposon locations for aminoglycoside resistance genes have been demonstrated (43, 53, 72, 73, 114, 131).

### Quinolones

The emergence of *Acinetobacter* spp. as important hospital pathogens has occurred at the same time as increased reliance on 4-quinolones for the treatment of serious infection. The development of 4-quinolone resistance is often quite difficult to demonstrate in the laboratory, and this finding has been extrapolated to suggest that resistance will be rare in the clinical situation. This is true for bacteria such as *E. coli* but does not seem to be the case for nonfermentative gram-negative bacteria such as *Acinetobacter* spp. Although the precise mechanism is virtually unknown, it is clear that *Acinetobacter* spp. can develop 4-quinolone resistance readily. Bacterial resistance to 4-quinolones in other genera has often been attributed to changes in the structure of the DNA gyrase subunits, usually by *gyrA* mutations. PCR has been used to amplify DNA surrounding the active-site region of the *gyrA* gene from 13 clinical isolates of *A. baumannii* with a range of ciprofloxacin MICs from 0.25 to 64 mg/liter (221). When the resulting PCR fragments were sequenced, it was found that the susceptible bacteria had 87 nucleotide differences, correlating with 13 amino acid differences, compared with the same 290-bp fragment from *E. coli*. The residues Gly-81, Ser-83, Ala-84, and Gln-106, whose substitution leads to 4-quinolone resistance in *E. coli*, were all conserved in the susceptible *A. baumannii* strains. All nine isolates of *A. baumannii* with ciprofloxacin MICs of >2 mg/liter showed a substitution of Ser-83 to leucine and Ala-84 to proline. Four strains with an MIC of 1 mg/liter did not show any change at Ser-83, but one exhibited a change from Gly-81 to valine. The results correlated with those from *E. coli* in that substitution of Ser-83 was responsible for, or at least contributed to, ciprofloxacin resistance in *A. baumannii*. *gyrB* mutations, causing changes in the  $\beta$ -subunit, occur less frequently in other bacteria and rarely result in such high levels of resistance. None have been examined in *Acinetobacter* spp.

*Acinetobacter* strains are less permeable to antibacterial agents than are many gram-negative organisms, and 4-quinolone resistance can also be conferred by outer membrane changes that result in decreased uptake. Selection of resistance by 4-quinolones can result in cross-resistance to  $\beta$ -lactams in *E. coli* and *P. aeruginosa* (134). This suggests that resistance results from alterations in the outer membrane, leading to decreased uptake. Studies on the development of 4-quinolone resistance in *P. aeruginosa* suggest that outer membrane protein changes are an early development in the buildup of resistance genes contributing to 4-quinolone resistance (154), and this is also likely to be true for *Acinetobacter* spp. In support of this hypothesis, an increase in the proportion of *Acinetobacter* isolates with combined resistance to all  $\beta$ -lactams, all aminoglycosides, and quinolones has been demonstrated (129).

### Other Antibiotics

Although it is known that various antibiotic resistance genes carried on plasmids of different incompatibility groups can be transferred into *Acinetobacter* spp. from *E. coli* (35), there have been few other studies of antibiotic resistance mechanisms in

clinical isolates of *Acinetobacter* spp. High-level (MIC, >1,000 mg/liter) trimethoprim resistance has been reported (34, 72, 129), and the genes encoding such resistance are often associated with multiple other resistance genes in transposon structures on large conjugative plasmids. Similarly, the chloramphenicol acetyltransferase I (CAT1) gene has been associated with both chromosomal and plasmid DNA in a clinical *Acinetobacter* isolate, suggesting that the CAT1 gene might be transposon encoded and had improved its survival potential by locating in both replicons (53).

### THERAPY OF ACINETOBACTER INFECTIONS

Very few of the major antibiotics are now reliably effective for the treatment of severe nosocomial *Acinetobacter* infections, particularly in patients confined to ICUs.  $\beta$ -Lactam antibiotics should be used only after extensive in vitro susceptibility testing has been performed. Ticarcillin, often combined with sulbactam (111, 212), ceftazidime, or imipenem, may be useful. Aminoglycosides can sometimes be used successfully in combination with an effective  $\beta$ -lactam, and other combinations of a  $\beta$ -lactam with a fluoroquinolone or rifampin have also been proposed.

In a retrospective survey in France of prescribing habits in 89 ICUs (102), the first-line therapy for *Acinetobacter* infections included amikacin, imipenem, ceftazidime, and/or a quinolone (pefloxacin or ciprofloxacin). In 56% of cases, imipenem was prescribed either as a single agent or in combination with amikacin (18%), while ceftazidime plus amikacin was prescribed in 17% of cases and amikacin was used as a single agent in 26% of cases. For second-line therapy after in vitro susceptibility testing, a combination of imipenem plus an aminoglycoside was used in 59% of cases, ceftazidime plus an aminoglycoside was used in 30% of cases, and ceftazidime combined with a quinolone was used in 11% of cases. A previous study (129) had shown that imipenem was used as monotherapy in 20% of 33 nosocomial infections, imipenem in combination with amikacin was used in 40%, and pefloxacin plus amikacin or tobramycin (depending on the antibiogram) was used in 20% of cases; treatment failure and death (caused by *Acinetobacter* infection and/or underlying disease) occurred in 17% of patients who received antibiotics. Many similar studies can be found in the medical literature (8, 29, 118, 214). In general, the recommended drugs in most recent studies have been either extended-spectrum penicillins, broad-spectrum cephalosporins, or imipenem, combined with an aminoglycoside.

### CONCLUSIONS

*Acinetobacter* spp. now account for a substantial proportion of endemic nosocomial infections. The relative importance of endemic and epidemic infections is difficult to delineate, but since about 50% of isolates are recovered from ICU patients and many such isolates correspond to epidemic strains, it can be estimated that about half of all infections are of epidemic origin. Recent trends indicate increasing antimicrobial resistance of *Acinetobacter* isolates (29, 94), posing a serious threat to hospitalized patients. Such outbreaks are often associated with multiple epidemic strains by sensitive typing techniques; however, multiresistant strains differing by only one or two antibiotic susceptibility markers are often shown to be closely related (70, 176, 191). For practical purposes, multiresistant strains of a similar antibiotype should be considered epidemic a priori unless proven otherwise.

The variety of potential sources of contamination or infection with *Acinetobacter* spp. in the hospital environment makes

control of outbreaks caused by these organisms one of the most difficult challenges in infection control. Outbreaks may result from intrinsic contamination of medical equipment and devices (e.g., respiratory equipment, intravenous catheters, or needles) used in patients for monitoring or therapy and/or from contamination of the environment, either by the airborne route or by contact with patients (e.g., mattresses, pillows, air humidifiers). Persistence of *Acinetobacter* spp. in the environment provides ample opportunities for contamination of patients and staff and may explain continuing long-term outbreaks. The emphasis of initial control measures should, however, be on strict isolation of infected or colonized patients to limit dissemination of outbreak strains in the environment. Isolation and cohorting of patients and staff has been shown to be effective in some outbreaks (56, 70, 145, 176), but this method alone has often been found to be insufficient to control outbreaks. It is useful to review hand-washing policy and practices, because cross-transmission via the hands of staff has been demonstrated in several outbreaks (67, 70). Concomitant to isolation precautions, an investigation of the dissemination of *Acinetobacter* strains in the environment should be undertaken, and if substantial contamination is found in the vicinity of infected or colonized patients, housekeeping practices should be reviewed and, if necessary, reinforced (39). In some instances, extensive measures, including closing the unit for complete disinfection, has been necessary (182, 191). In one outbreak, even this measure was not sufficient, because of persistence of the epidemic organism on contaminated equipment in the unit (182). Case-control studies are appropriate to find a common environmental source to which patients have been exposed. When exposure to a particular item of equipment is implicated, it is necessary to determine whether extrinsic contamination has occurred during use in infected or colonized patients or whether intrinsic contamination (e.g., via ineffective sterilization or contamination by staff carriers during handling) is the cause (80).

There is some evidence that increased use of antibiotics favors the emergence and spread of *Acinetobacter* spp. (29, 94). An outbreak of imipenem-resistant *Acinetobacter* infections following increased use of imipenem in response to an outbreak involving cephalosporin-resistant *Klebsiella pneumoniae* has been described previously (70). Multiresistant *Acinetobacter* spp. are likely to be selected in the hospital environment in response to increasing antibiotic pressure. Control of antibiotic usage is therefore also an important part of preventive measures against the emergence of epidemic *Acinetobacter* infection.

*Acinetobacter* spp. are increasingly important nosocomial pathogens and are capable of rapid adaptation to the hospital environment. There is no doubt that these organisms will pose continuing problems in the future, which is disturbing because of the extent of their ever-increasing antibiotic resistance profiles. A combination of control measures is often required to contain these organisms. Continued awareness of the need to maintain good housekeeping and control of the environment, including equipment decontamination, strict attention to hand-washing and isolation procedures, and control of antibiotic usage, especially in high-risk areas, appears to be the combination of measures most likely to control the previously unabated spread of *Acinetobacter* spp. in hospitals.

#### ACKNOWLEDGMENTS

We are indebted to numerous colleagues for contributing to our understanding of the biology and role of *Acinetobacter* spp. as nosocomial pathogens over many years.

Work in our laboratories on *Acinetobacter* spp. has been supported by grants from the French Ministry of Health, the U.K. Trent Regional Health Authority, Roussel, Eli-Lilly, Glaxo, and Merck-Sharp & Dohme Laboratories.

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